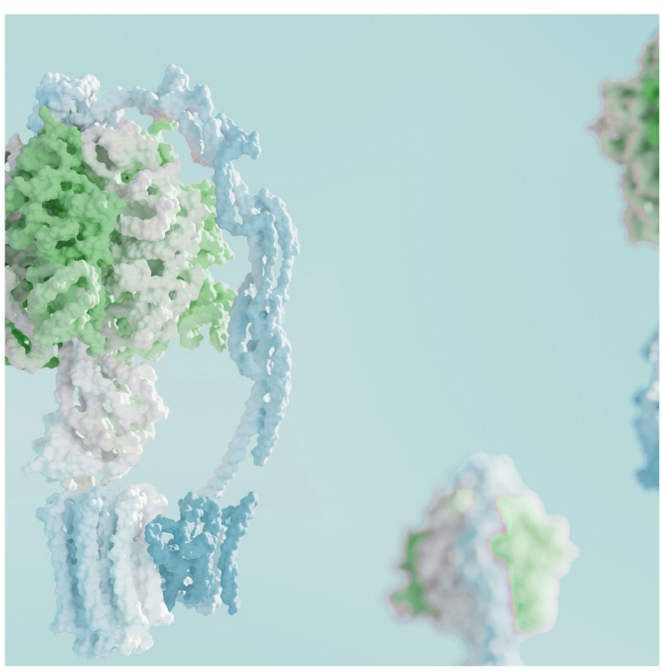
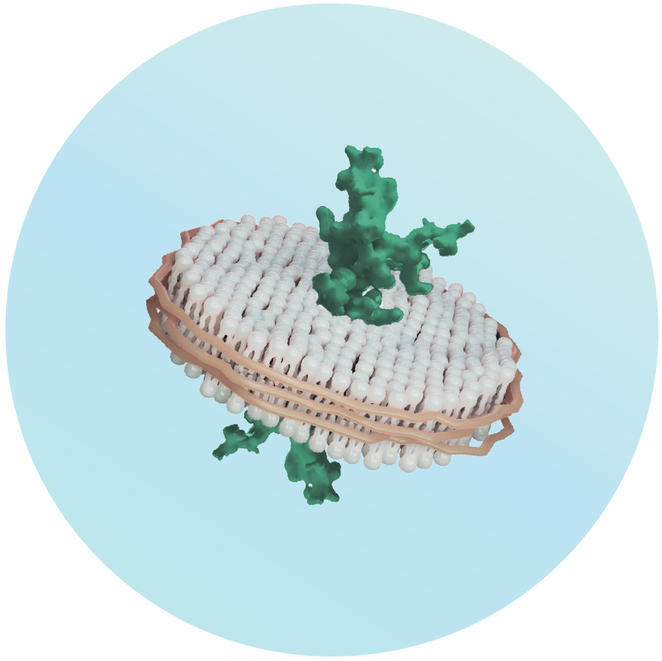
SERVICE
BROCHURE



Cube Biotech

INTRODUCTION



As a CRO team of passionate (membrane) protein scientists, we offer non-GMP protein services for a broad range of applications. From custom protein expression services to characterization and cryo-EM protein structure determination, everything is possible. Individual work packages made up of our modular structure can be tailored to your specific needs.

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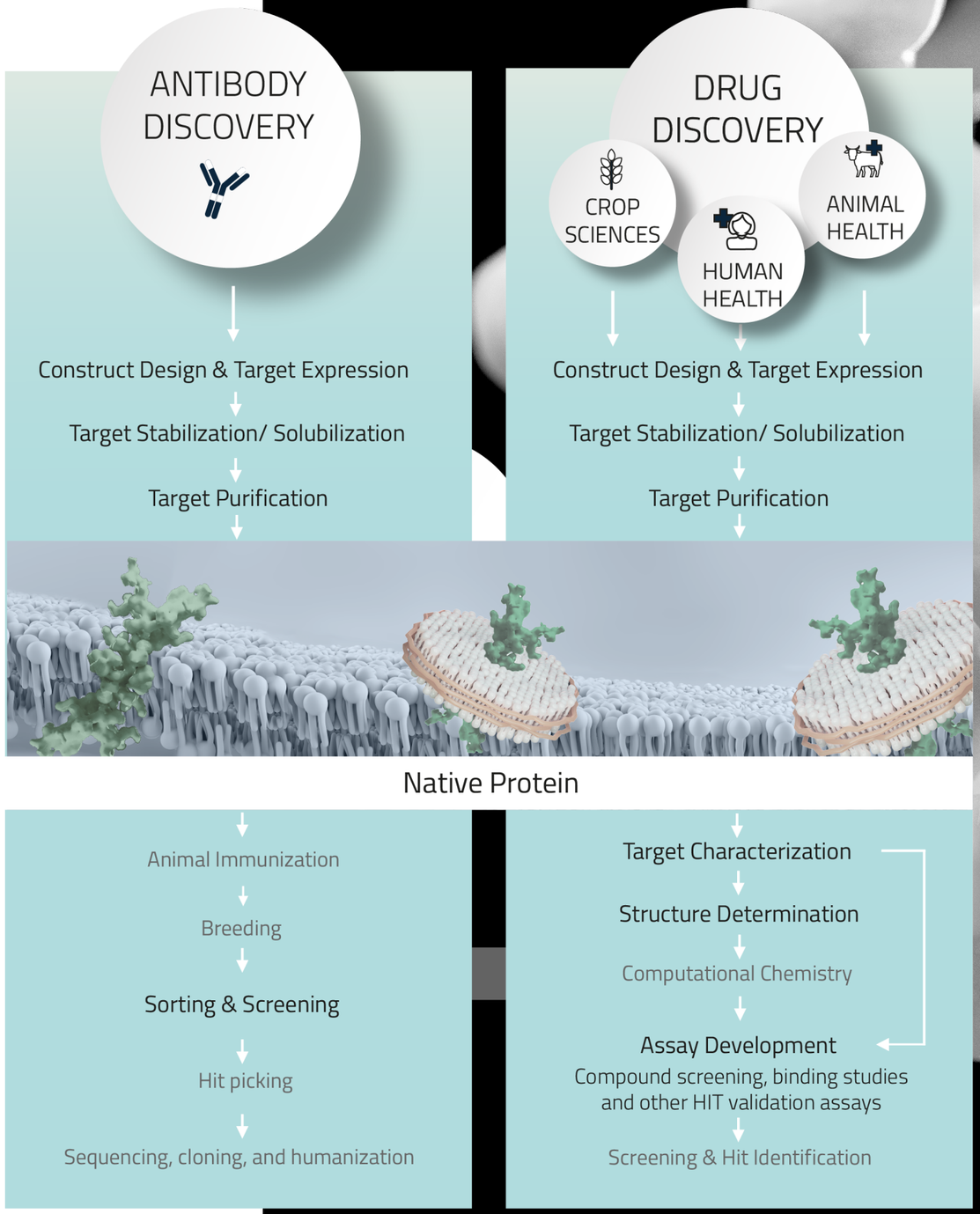
Service SEGMENTS

The team at Cube Biotech offers solutions for many fields of protein science: Drug discovery in Crop Science, Animal or Human Health as well as Antibody Discovery.

For issues in those categories, Cube Biotech can help in all stages of protein preparation, but also with target characterization (by biochemical or biophysical methods), and structure determination by cryo-EM and crystallization or assay development.

The figure highlights the work processes in the individual segments that we can undertake on your behalf.



Drug DISCOVERY

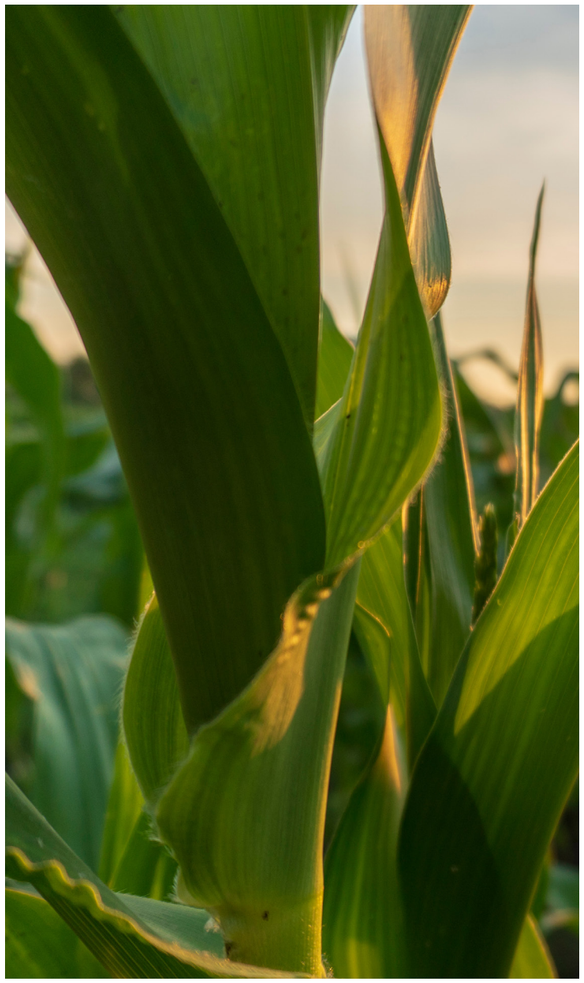
The proper target characterization and validation are key factors of the drug discovery pipeline.

Many therapeutically interesting targets are membrane proteins like GPCRs, ion channels, or receptors, which are notoriously difficult to handle and not easily characterized in their native state. For a bottom-up or top-down approach to small molecule or biotherapeutic discovery the target needs to be purified to fully characterize the structure and function.

The discovery of new drugs for human, and animal health as well as in the field of crop science is a necessity to fight diseases and growing resistance in various pest types.

Here, a clear connection between structure and function is absolutely important to gain knowledge about how a drug binds to its target and how the mechanism is altered.

One example to highlight that the 3D-structure of an apo- and ligand-bound molecule elucidates the structure-function, is Slo1. Slo1 is an insect specific potassium ion channel which undergoes conformational changes while binding fungal neurotoxin Verrulogen and the anthelmintic drug Emodepside.



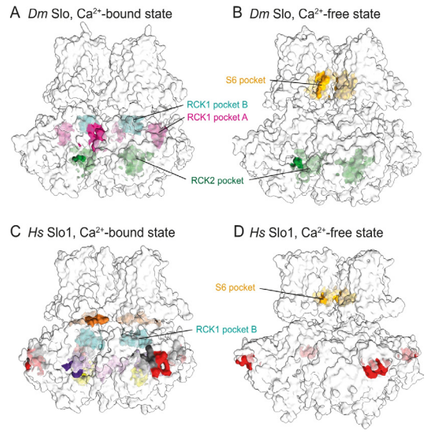
DEVELOPING ECO-FRIENDLY PESTICIDES

We teamed up with Bayer Crop Sciences and the Max Planck Institute for Molecular Physiology to pave the road for the production of eco-friendly and bio-degradable pesticides.
The idea was to destroy the nervous system of harmful insects by attacking the insect-specific homolog of the potassium ion channel named Slo1.

Two substances called Verruculogen and Emodepside were suspected to only bind to the insect homolog of Slo1 specifically without an off-target binding to the human or plant homolog.
To gain this knowledge extensive research into the 3D-structure of the Slo1 protein was conducted to explore the conformational and activity changes after Verrulogen or Emodepside binding.

Many cryo-EM measurements were performed for structural elucidation, and Cube Biotech provided the researchers with plenty of recombinant Slo1 protein for their projects.

It was of utmost importance that the membrane protein samples were of the highest purity and homogeneity for e.g. the cryo-EM measurements.



The human homolog of Slo1 (Hs C and D) was compared to the *D. melanogaster* homolog of Slo1 (Dm A and B) to ensure that ligands like pesticides only bind to one of them and not the other (Raisch *et al.* 2021).

THE PROJECT'S TASKS

- Transfection of *T. ni* insect cells with the Slo1 gene containing plasmid
- Optimization and conduction of recombinant Slo1 membrane protein expression
- Screening for the best-suited detergent for Slo1 membrane protein solubilization
- Stabilization of Slo1 membrane protein in detergent micelles
- Purification of Slo1 membrane protein using Rho1D4 magnetic beads with addition of insect cell lipids

Antibody DISCOVERY

For immunization and antibody production purposes, usually, a huge amount of high-quality protein is needed: the antigen. This antigen can be injected into animals that produce antibodies against the unknown structure.

Cube Biotech offers great experience in recombinant expression and purification of antigens for soluble and membrane-bound target proteins to help scientists achieve their goals in the field of antibody discovery.

In this example, we'll concentrate on the tale of one of our more unique projects - the isolation of the acetylcholine-receptor AChR from the electric ray *Torpedo californica*.

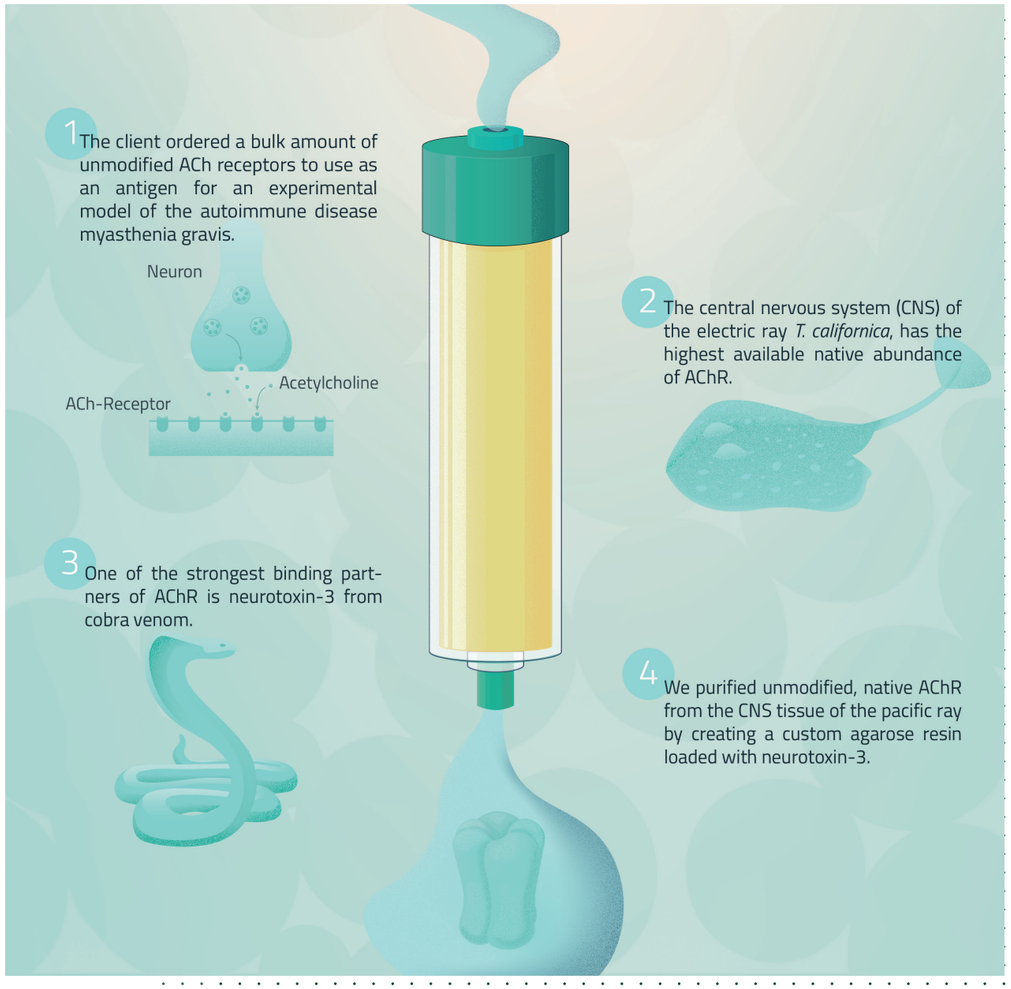
Our client needed a bulk amount of unmodified AChR to use as an antigen for an experimental model of the autoimmune disease myasthenia gravis.

This chronic muscular disease is caused by the degradation of the AChR by IgG autoantibodies (Losen *et al.*, 2015). That leads to a weakness of voluntary muscles and thus to an impairment of body movement and breathing.

The recombinant production of native-like, functional heteropentameric AChR is not possible which also excludes the utilization of affinity tags. Getting a high protein yield without modifying the protein was a challenge. Our innovative solution was to use the central nervous system (CNS) of the electric ray *T. californica*, which has the highest available native abundance of AChR.

As affinity tags were not an option, we decided to use the protein's inherent affinities.

One of the strongest binding partners of AChR is neurotoxin-3 from cobra venom. Hence, we purified unmodified, native AChR from the CNS tissue of the pacific electric ray using the ligand neurotoxin-3 covalently coupled to our agarose resin beads. We followed a slightly altered version of the Losen *et al.* (2015) methodology to optimize the purification. This is an excellent demonstration of how we combined our protein and custom bead services to meet our client's objectives. The preparation of *Torpedo californica*'s AChR is not an example of a classical antibody generation process, however, shows the broad spectrum of methods Cube Biotech is capable of providing to make our customer's antibody discovery projects a great success.



Start your PROJECT

We can build a framework for your project, which can be seamlessly integrated into your own workflow of the research and development program.

It is extremely important to us that the service we provide meets your requirements. Thus, each project is tailored to your protein of interest, whether, you work on a soluble or membrane protein. For us it is a matter of course to react rapidly and reasonably to obtain data and, if necessary, choose a different strategy in the interest of the project to achieve your goals. Working with proteins requires having a plan B or even C before the project starts since each protein is unique and there is no one-fits-all solution.

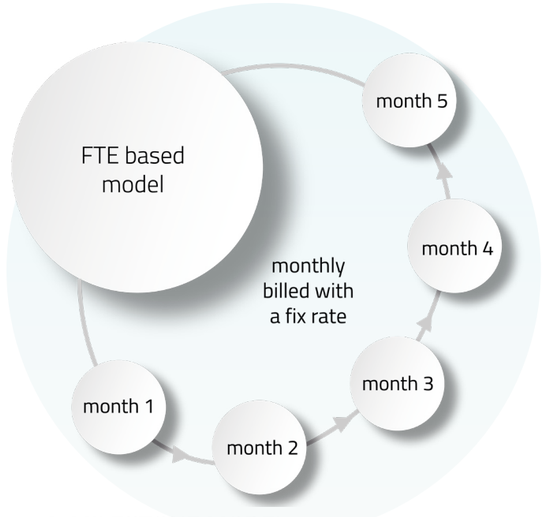
We practice a comprehensible and transparent way of sharing received data, results, and documentation. Upon request you can receive a technology transfer such as an elaborated protocol, to perform the work in your laboratory whenever needed. If you would like to sign a CDA first, this is feasible.

WANT TO LEARN MORE?

Just contact us to arrange a meeting.

THREE PROJECT MODELS

We have gained a profound knowledge of various targets (membrane protein, soluble protein, and peptides) in the past decade, which showed that screening is the key to success and to providing pure protein of the highest quality. Depending on your preferences, and project goals, we offer three different project models:

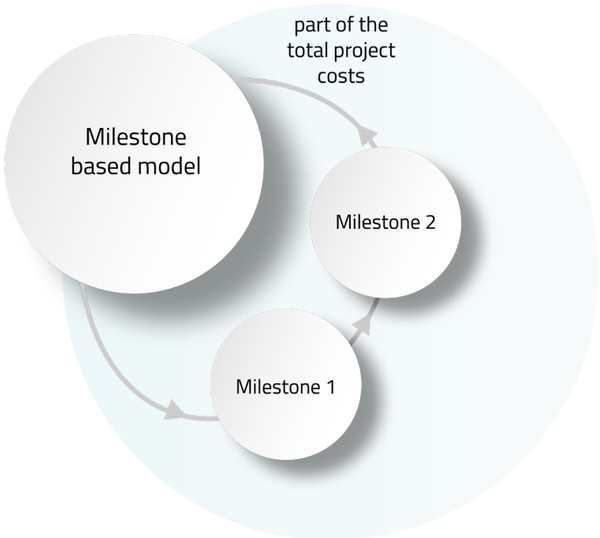


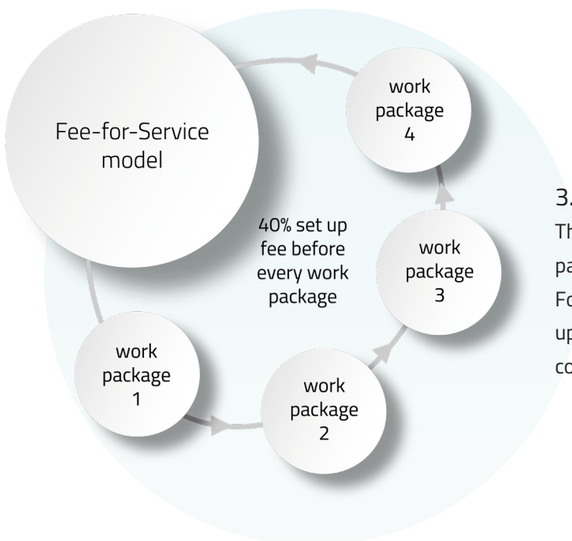
1. FTE (FULL TIME EQUIVALENT) MODEL

This model delivers the highest flexibility in terms of scientific work. It is particularly well suited for projects with unknown or less studied targets. In this project model, you are billed monthly with a fixed rate.

2. MILESTONE MODEL

In this project model, the customer benefits from increased flexibility in terms of scientific work in combination with a lower risk in case the project fails. The milestone-based model is also structured in work packages. You are charged with a part of the total project costs as an upfront payment as soon as the project starts and the next payment is due as soon as determined milestones are fulfilled.





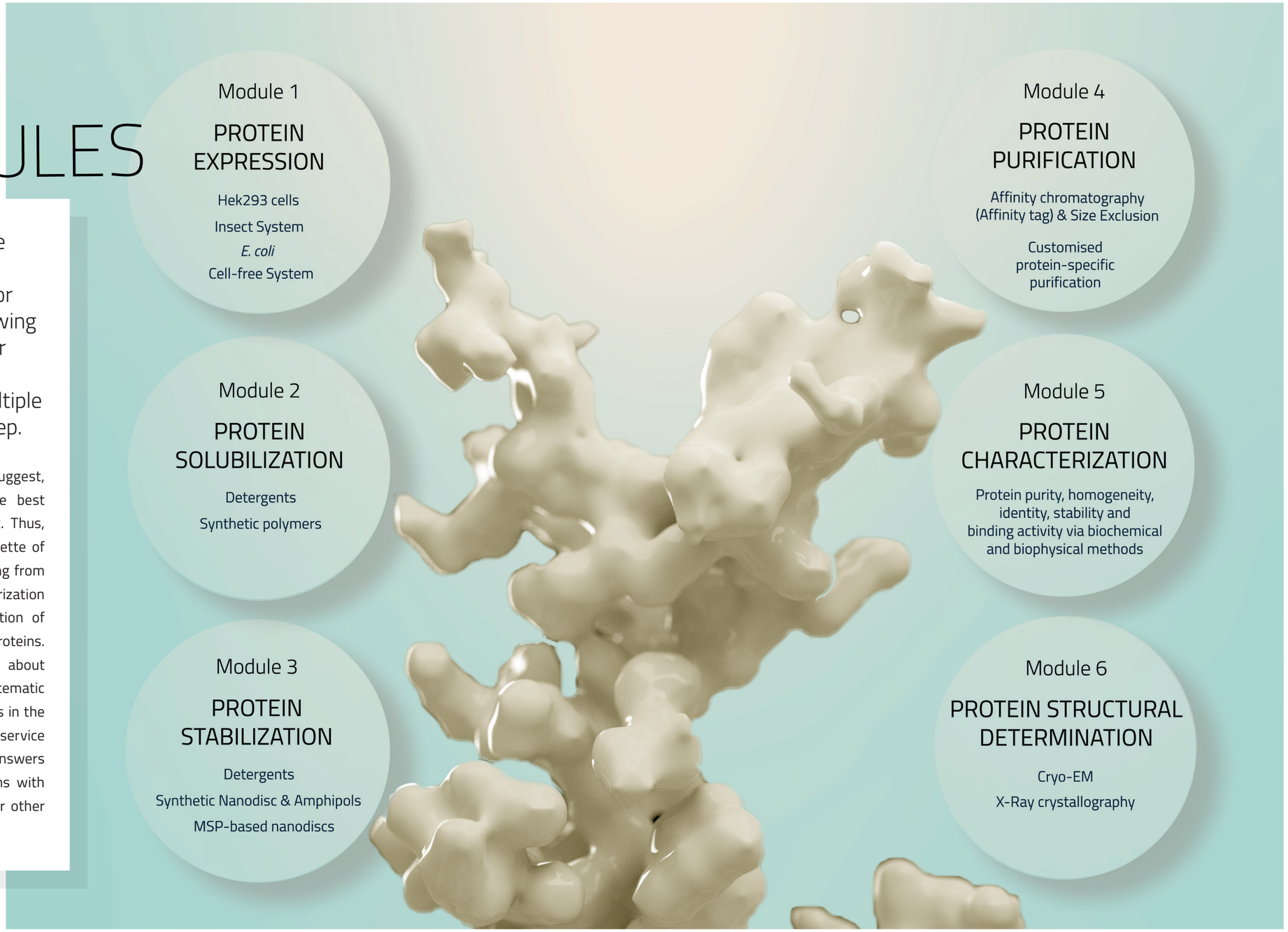
3. FEE-FOR-SERVICE MODEL

This project model is structured in different work packages and is especially suitable for smaller projects. For each work package, you are charged a 40% set-up fee before the work package starts and a 60% completion fee as soon as the work package is finished.

Our MODULES

Our protein service packages offer modular options for project tasks, allowing you to choose your starting point and approach with multiple options at each step.

Our service goal is to suggest, optimize, and deliver the best workflow for your project. Thus, we offer an extensive palette of tools and methods covering from expression to characterization and structural determination of soluble and membrane proteins. Based on discussions about your needs and a systematic examination of approaches in the literature, we assemble a service proposal that delivers the answers to your research questions with options to meet budget or other constraints.



Module 1

PROTEIN EXPRESSION

Hek293 cells

Insect System

E. coli

Cell-free System

Module 2

PROTEIN SOLUBILIZATION

Detergents

Synthetic polymers

Module 3

PROTEIN STABILIZATION

Detergents

Synthetic Nanodisc & Amphipols

MSP-based nanodiscs

Module 4

PROTEIN PURIFICATION

Affinity chromatography (Affinity tag) & Size Exclusion

Customised protein-specific purification

Module 5

PROTEIN CHARACTERIZATION

Protein purity, homogeneity, identity, stability and binding activity via biochemical and biophysical methods

Module 6

PROTEIN STRUCTURAL DETERMINATION

Cryo-EM

X-Ray crystallography

MODULE 1

Protein EXPRESSION

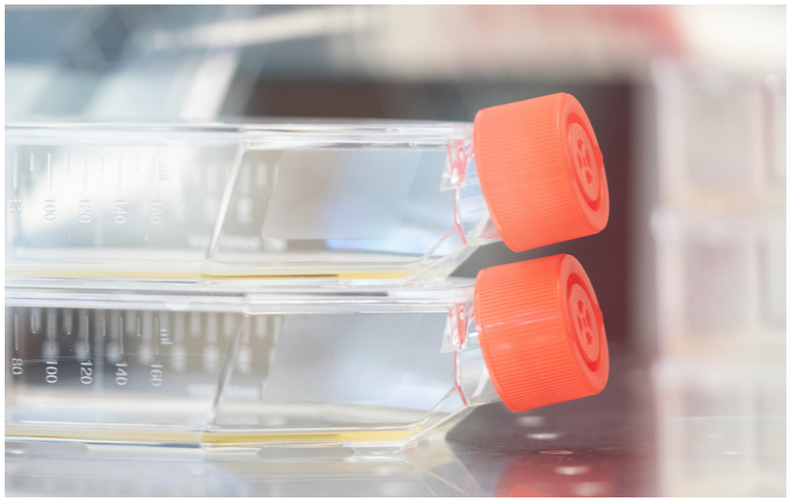
We typically start with sequence-optimized, full-length genes expressing a wild type protein. In accordance with the project's requirements, different constructs with different affinity tags and positions as well as truncations of point mutations can be included, if desired.

We are optimizing the protein expression to the fullest. Next to the expression systems, features that we optimize for maximum protein yield are:

- Expression conditions (media, temperature, induction conditions)
- Promoter regions (strong/weak expression, tightness of control)
- Type and position of the affinity purification tag
- Optional: best-expressing domains, including a boundary screen of domain borders

After discovering the ideal expression conditions for your protein of interest, we can scale up the expression.

This way we can produce larger amounts of your protein in a shorter time period.



HEK293 CELLS

Advantages: Homolog mammalian protein expression
Amount of purified protein: Lowest of the four, but with the highest authenticity among our systems

BACULOVIRUS INSECT SYSTEM

Advantages: Near mammalian-like post-translational modifications; easy to handle eukaryotic expression system
Amount of purified protein: Higher than mammalian cell lines

E. COLI EXPRESSION *IN VIVO*

Advantages: Fast and simple production of recombinant proteins; most suitable for soluble targets
Amount of purified protein: Highest among our expression systems

E.COLI BASED ON CELL-FREE EXPRESSION

Advantages: Direct stabilization and co-translational integration of membrane proteins into nanodiscs; useful for toxic proteins
Amount of purified protein: Less than in *E.coli in vivo*, but stabilized during expression

MODULE 2 & 3

Solubilization & STABILIZATION

Working with isolated, purified, and especially functional membrane proteins requires protein solubilization and stabilization to build the most comfortable environment for the target membrane protein.

To identify this one specific condition and solubilization or stabilization reagent for each unique membrane protein, extensive screening work and established platform technology are needed.

In this context, we offer a huge toolbox consisting of detergents, synthetic copolymers, and MSP-based nanodiscs that will be used for screening during our service projects based on your preferences.

DETERGENTS

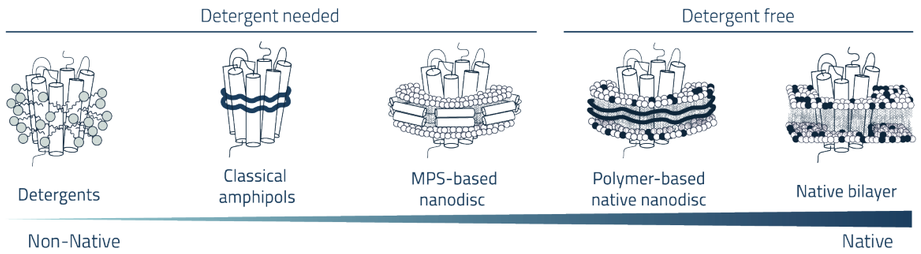
The traditional way to solubilize membrane proteins. We offer a large selection of different detergents, which are well-known and described.

MSP-BASED NANODISCS

These protein-based nanodiscs have several advantages over traditional detergent-based approaches for membrane protein stabilization. With their freely selectable phospholipid composition, a controlled environment that mimics an *in vivo* situation can be created. Or a specific interaction between membrane protein and phospholipid can be studied. Therefore, a solubilization step in detergents is needed first. The handling of nanodiscs, in general, is one of Cube Biotech's strongest assets. Years of unmatched experience make Cube Biotech the best option for MSP-based nanodisc-related projects.

SYNTHETIC POLYMER-BASED NANODISCS

As an innovative option, synthetic copolymers have the capability to solubilize and stabilize membrane proteins in a single step. The membrane protein remains surrounded by the lipid composition derived from the expression host and is belted by a synthetic copolymer: a synthetic nanodisc is built. For this technique, detergent solubilization is not needed at all. Utilizing synthetic polymers will leave the original lipid composition of the membrane protein of interest intact and this has an effect on the activity and stability of the membrane protein complex.



DISCOVERING OPTIMAL SOLUBILIZATION AND STABILIZATION CONDITIONS

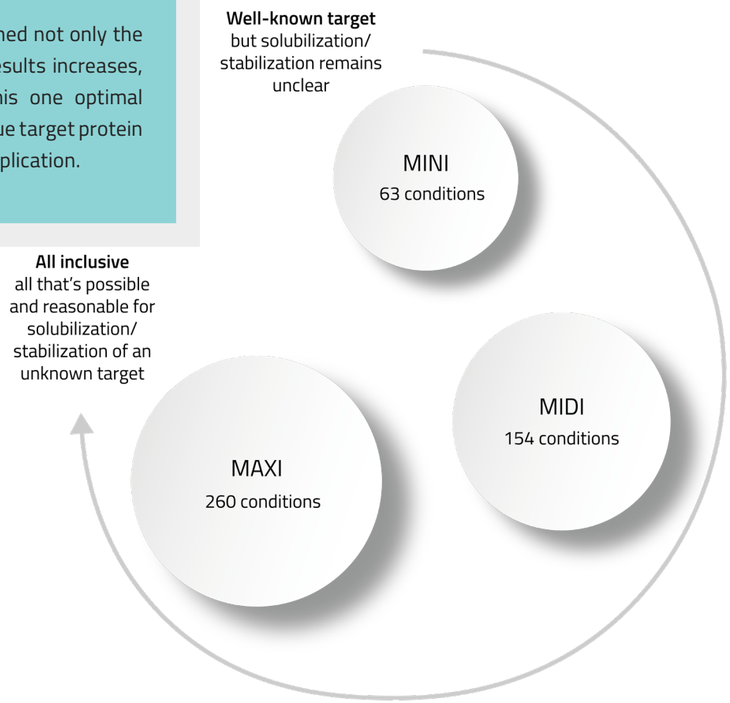
For a service project, the customer can choose between a MINI, MIDI, or MAXI screening approach. The choice of screening scope and substances depends on several aspects: characterization of the membrane protein in literature, the intended downstream application, timeline, and budget. No matter which substances will be used during the screening (detergents or synthetic polymers) the amount of conditions screened is as follows:

MINI: 63 conditions
MIDI: 154 conditions
MAXI: 260 condition

As more conditions will be screened not only the chance for positive or suitable results increases, but also the chance to find this one optimal condition for the customer's unique target protein and the intended downstream application.

Variation of these conditions:

Reagent
Concentration
Temperature
Time
Source



MODULE 4

Protein PURIFICATION

Protein purification aims to isolate a single target protein from a biological tissue or culture. It is applied to produce proteins for scientific research purposes, like drug and antibodies discovery, etc. Therefore, protein purification techniques are based on the exploitation of differences in the properties of the target protein and other proteins such as e.g. ligand-binding affinities, size, charge, solubility, or hydrophobicity.

To extract the desired protein from the rest of the cell's components after cell lysis and stabilization, in the case of a membrane protein, Cube Biotech offers numerous purification techniques and often multiple are combined.

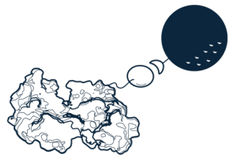
An ideal protein purification strategy is one in which the highest level of purity is achieved in the fewest steps to avoid high protein loss and thereby higher costs.

Our standard purification methods are Affinity Chromatography, Ion-Exchange Chromatography (IEC), Hydrophobic Interaction Chromatography (HIC), and Size Exclusion Chromatography (SEC).

Besides affinity purification via columns, we, as a manufacturer, have the possibility of working with magnetic beads on a larger scale. In this way we are able to process large amounts of lysate quickly and gently in order to elute the proteins in small farctions. This avoids lossy concentration steps.

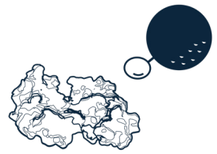
For further information on these techniques, please follow this QR code to watch our video on protein purification (https://www.youtube.com/watch?v=KOTNXKo2O7o).

For Affinity Chromatography and with regard to membrane proteins, our favorite affinity tag for purification is the Rho1D4 tag. Since it is an antibody-based affinity tag it provides incredible specificity and high yields. We are, however, open to discussing and using other affinity tags or tag combinations in your project if you like.

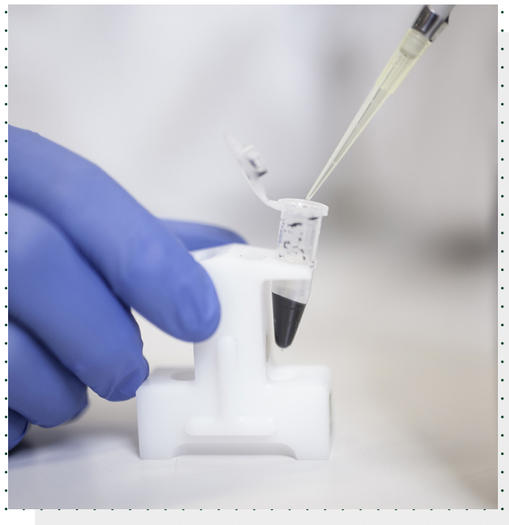


Surface affinities/ affinity tags

Additionally, we can produce a specialized protein purification matrix just for you on request. It can include a protein-specific antibody, a natural ligand of the protein of interest, or other components specialized for your needs.

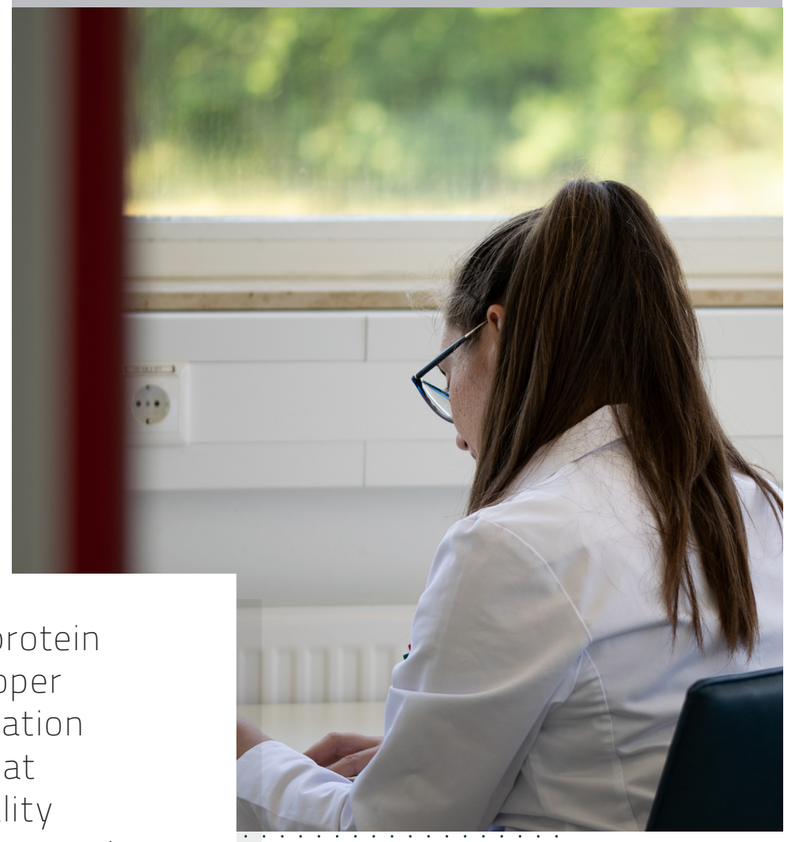


Customized agarose resins/ magnetic beads



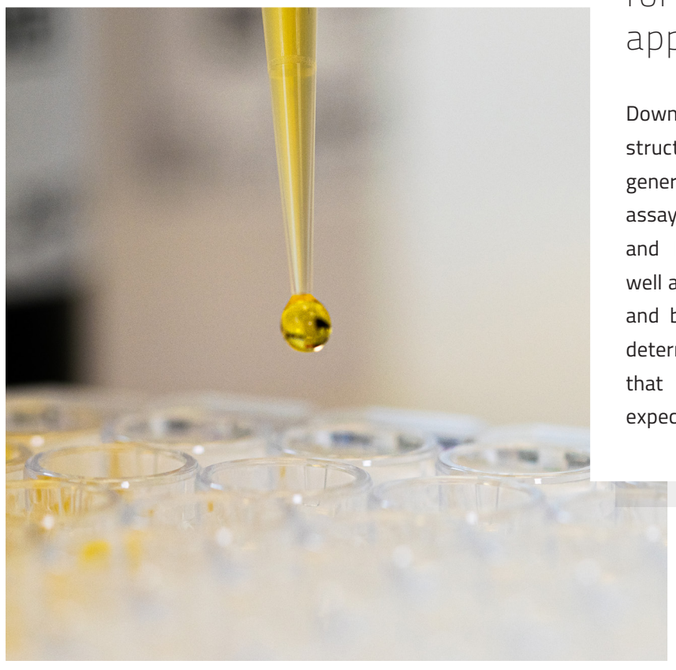
MODULE 5

Protein CHARACTERIZATION



A purified protein needs a proper characterization to show, that certain quality attributes are met for the downstream application.

Downstream applications involves i.e. structural determination, antibody generation, function activity assay, binding assay, etc. Quality attributes are purity and homogeneity, identity, activity as well as stability. A selection of biophysical and biochemical methods enables us to determine specific parameters to show that the purified protein is within the expected range for a high-quality sample.



DYNAMIC LIGHT SCATTERING (DLS):

A method to determine the hydrodynamic radius and the particle size distribution of your protein sample by measuring how light is scattered depending on size.

PROTEIN STABILITY ASSAYS:

We can determine the stability of your protein under specific conditions: The purified protein is applied to freeze/thaw cycle testing, thermostability testing (storage at 2-8°C, RT, accelerated aging temperature > 24 °C); conformation stability testing (thermal ramp up to 95°C via nanoDSF), colloidal stability testing (thermal ramp up to 95°C via DLS device).

SURFACE PLASMON RESONANCE (SPR):

A method to determine kinetic parameters of binding between for example two proteins or protein-ligand, antibody-antigen by the change of the refraction index depending on mass change at the plasmon.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA):

Assay to identify the reaction of an antibody to an antigen (target protein). This allows determining the amount of antigen or antibody found in a biological sample (i.e. blood sample) depending on how the assay is designed.

Biophysical and biochemical methods for target protein characterization

	Protein Specific Activity ssay	ELISA	SPR	MST	aSEC (apparent MW)	SDS-PAGE	Blue native Page	Western Blot	DLS	NanoDSF
Activity										
Identity										
Purity and Homogeneity										
Stability										

MODULE 6

Protein Structure DETERMINATION

One of the key characteristics of a protein is its 3D structure, it is also one of the hardest characteristics to identify.

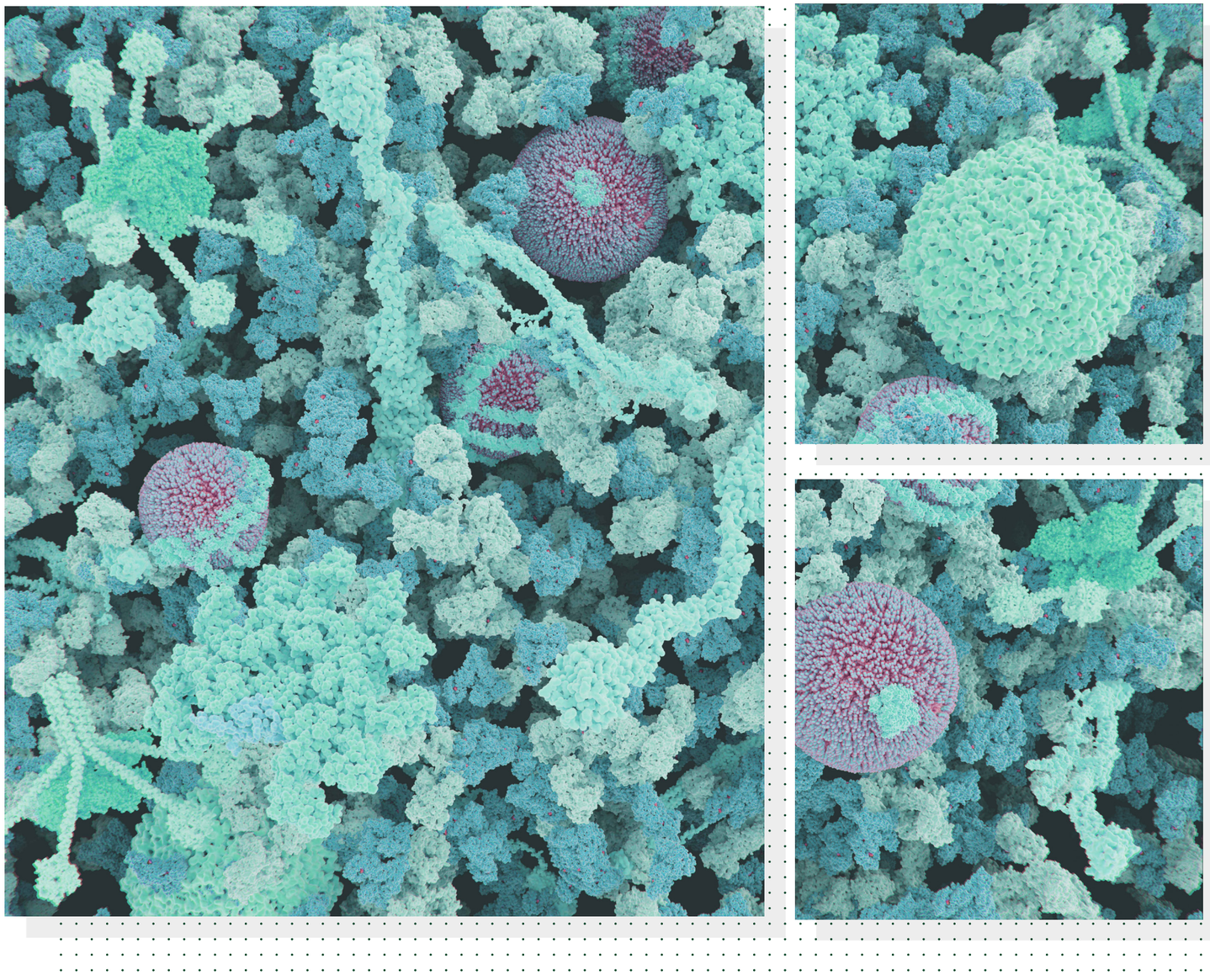
Cube Biotech offers two structure determination methods for your membrane or soluble protein. The generated high-resolution structures of your target protein with a potential drug candidate will facilitate identifying and optimizing a hit to a lead structure by structure-based drug design (SBDD).

1. CRYO-EM:

Cryo-EM is the state-of-the-art method for protein structure determination for protein and protein complexes > 100 kDa. Especially for membrane proteins, cryo-EM is the most plausible option to identify their atomic structure as other methods fall short on the lead time and the high sample requirements.
We offer a modular cryo-EM service, ensuring a seamless handover of protein production to grid preparation/screening, data collection, and atomic modeling of the structure. The workflow will enable us to generate the structure of protein-small molecule/peptide, protein complexes, and protein nucleic acid complexes. We have industry access to a larger cryo-EM facility accommodating the project timelines.

2. X-RAY CRYSTALLOGRAPHY:

Our services allow us to gain high-resolution structural information on soluble proteins and membrane proteins by the classic approach of X-ray crystallography. One way to solve 3D structures of membrane proteins in a lipidic environment is the Lipid Cubic Phase (LCP) crystallization. Our patented CIMP (controlled in-meso phase crystallization) method combines LCP crystallization and vapor diffusion for faster and easier handling of the crystallization process.
We offer a modular service of crystal screening, crystal hit optimization, data collection, data processing, and atomic structural determination of your target protein. Measurements are planned regularly with access to European synchrotrons (i.e. DESY).



FUNCTIONALIZED Particles

Cube Biotech is committed to delivering customized particle functionalization services that precisely match your specifications and address the unique functionality for targeted applications. With the extensive expertise of our experienced researchers, we tailor solutions according to your specific requirements.

For this, we utilize particles made of different materials, shapes, and sizes. We offer controlled functionalization even with a defined number of biomolecules attached to the particle and provide expertise in the conceptual design of particle constructs.

Our service spectrum encompasses particle functionalization with small molecules, proteins, protein-specific antibodies, and nucleic acids, as well as materials ensuring stability in common buffer systems and offering versatility and adaptability across various applications.

Moreover, our state-of-the-art physicochemical characterization ensures high-quality particles at every stage, from proof-of-principle lab-scale development to production on an industrial scale.

In addition to the established classics like PureCube Agarose Beads and PureCube MagBeads, we boast an array of other base materials to meet your particular scientific needs. Nanoparticles made of different noble metals (e.g., gold, silver, platinum), characterized by their unique optical and physical characteristics, offer immense potential for innovation in R&D, including (bio)imaging, protein labeling in TEM, lateral flow assays, etc. For additional details, please explore our website www.cube-biotech.com.

TYPE	MAGBEADS	AGAROSE	GOLD NOBLE METALS
SIZE & SHAPE	30 µm – 800 µm Beads	40 µm – 800 µm Beads	5 nm – 100 nm Sphere | Rod | Cube
BASE MATERIALS	Agarose Beads with distributed Ferrimagnetic Particles	Agarose Beads*	Gold (with selected coating)** | single-molecule functionalization possible
COUPLED MOLECULES & LIGANDS	Ligands | Natural Ligands | Drugs | Carbohydrates | Cholesterol Derivatives | Amino Acids | Proteins | Peptides | Antibodies Click reagents | Fluorophors and Colorants | Activation Group		Nucleic Acids Antibodies Fab-fragments | Fluorophores Biological Tags (e.g. Affinity-Tags) Polymers | Small Molecules
APPLICATION AREAS	Protein-specific Purification and Pulldown (Membrane) Protein Purification Purification of Carbohydrates Immobilization of DNA/RNA/Oligonucleotides Electron Transfer Reagents in Fuel Cells | Molecule Depletion		**Imaging** X-ray Imaging Fluorescence Photoacoustic Imaging Optical Imaging **Diagnostic research** Nucleic Acid Detection Protein Detection e.g. Lateral Flow Assay

*Others on demand, e.g.: Hollow Agarose, Organic Polymers, Spherical Cellulose/ Carbohydrates
**On demand other noble metals such as silver and platinum possible

Options of particles with our functionalization services

About US

Unleashing the power of proteins for research and progress, Cube Biotech supports the pharmaceutical and biotechnical community with all its scientific expertise.

By leveraging the latest technologies and techniques, we are able to provide a comprehensive range of products and services that address the full spectrum of protein research.

CUBE BIOTECH INC. USA

From our office in Wayne, PA, Cube Biotech Inc. manages order fulfillment and inventory and provides a variety of distribution channels in the North American life science, biotech, and pharmaceutical markets.

RESEARCH & DEVELOPMENT

Our R+D and production site is located in Monheim, Germany, close to Cologne. We supply customers from here worldwide and also interact closely with our distributors and OEM partners.

Being a young and dynamic team, we are eager to open up new fields of research, to grow as scientists and as people, and to provide you with the highest quality of products.

A great passion for the life sciences and the opportunities they hold is what binds us together. Be a part of this idea and perform your research with quality products from Cube Biotech, because proteins are our passion.

SERVICE BROCHURE

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